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(54) SUSTAINED RELEASE OPHTHALMIC PHARMACEUTICAL PREPARATIONS CONTAINING COLLAGENS

(71) We, MEIJI SEIKA KAISHA LTD., and NIPPI, INCORPORATED, both Corporations organized and existing under the laws of Japan, of 8, 2-chome, Kyobashi, Chuo-ku, Tokyo, Japan, and 1-1-1, Senju Midoricho, Adachi-ku, Tokyo, Japan, respectively, do hereby declare the invention, for which we pray the method by which it is to be performed to be particularly described in and by the following statement:—

This invention relates to a collagen-containing ophthalmic pharmaceutical preparation having a slow releasing property and a process for preparing the same. Particularly, it is concerned with an ophthalmic pharmaceutical preparation or composition having a slow releasing property which comprises a solubilized collagen or a collagen "fiber" dispersion, and one or more of ophthalmic drugs and a process for preparing the same. More particularly, this invention relates to an ophthalmic therapeutic composition prepared with collagens, which has such excellent property that it can control the release of a drug constituted in said composition as an active ingredient for a prolonged period of time when applied therapeutically the collagen compositions having a drug incorporated therein and to a process for preparing such composition.

In medical treatment of diseases, drugs do play a prominent role, whereas side effects caused by these drugs have often given rise to several troubles. It is essential for an effective action of a drug, as well as with less side effect, to make the drug effectually available to the tissues or organs to be acted (hereinafter referred to frequently as "target"). However, a drug, when given through such an administration route as oral one, is absorbed in gastrointestinal tracts and transported to blood therefrom, finally to the target, and during the transportation the drug is often liable to be decomposed or metabolized. Moreover, the drug introduced in blood is absorbed through blood vessels into an entire body and then carried to or distributed to even other tissues and organs in an entire body besides the target, which frequently results in occurrence of undesirable side effects and availability of only a minor amount of the drug at the target as compared with a given dose. A far greater amount of a drug should, therefore, be given in oral administration than that required for the concerned target. In these circumstances, if a direct administration solely to the target is possible and an administered drug in required amount may sustain its action for a given period of time, it may be possible to avoid any side effects of drugs above-mentioned and to serve as an ideal medicament. Many of therapeutic agents against eye diseases are generally applied in the form of an ophthalmic solution or ointment. Such a treatment may be satisfactory for those eye diseases for which an application of a drug is required only once or twice. However it is inconvenient to those patients who are to be treated for their eye diseases with higher frequency of administration. Also, in case of ophthalmic drops as shown in the prior art, a major portion thereof is prone to flow out with tears and only a minor portion of the applied amount thereof may effectually act on as a medicament. The present inventor have made a research in attempts to avoid such disadvantages and have found the following facts that when a drug may be formulated into an ophthalmic preparation using collagens as a support for the drug as in this invention and inserted it into cul-de-sac in eyes, a slow release of the drug therein is possible and also it becomes practicable to utilize the drug effectively with full duration of action of the drug as well as with the minimum effluence through nasal lacrimal ducts. Furthermore, it is another effect of this invention that collagens are completely dissolved in and flew out with lacrima and not necessary to be withdrawn from eyes. Collagen is a principal protein which constitutes connective tissues of animals and the majority thereof is the one named as insoluble collagen.

The collagen employed in this invention is, as set forth hereinabove, solubilized collagens and collagen "fiber" dispersions and may be prepared as will be described below.

The above-mentioned insoluble collagen may be solubilized in water by a method comprising solubilization by treatment with a protease in such a way that the basic structure of collagen molecules is kept intact (Japanese Patent Publications No. 13871/1962 and No. 14426/1962 and British Patent 1,119,432), or a method wherein the insoluble collagen is treated with an amine, an alkali and sodium sulfate while retaining the basic molecular structure of the collagen (British Patent 1,192,314). In any of these methods, collagen molecules solubilized in water keep the basic molecular structure intact while the terminal telopeptide chains of the molecule are cleaved. Such type of collagens has characteristics of being low in immugence, favourable in compatibility with tissues of a living body, free of an inflammatory response and slowly absorbable in tissues. On the other hand, collagen is a protein which shows satisfactory properties of being retentive of various types of drugs and, further, of being low in immunity when applied as a supporting agent for drugs to be administered under controlled conditions of slow-releasing. Insoluble collagens are treated with an aqueous solution of an amine, caustic soda and sodium sulfate for 5 hours to 10 days, the treated collagens are desalted by washing with water and then adjusted to pH 3.5—9.5 and finally ground mechanically to yield a collagen "fiber-bundle" dispersion. The dispersion is treated with glutaraldehyde and then again ground mechanically at pH of 2.0—4.0 or 9.0—11.0 to yield a homogenized "fiber" dispersion.

The terms "fiber-bundle" and "fiber" are used to express the following matters, respectively. Linear arrays of collagen molecules are shifted longitudinally by about one-fourth of the molecular length to form fibrils (minute fiber). Fibrils may vary in its diameter depending upon tissues and host animals, but they have a diameter of 1000—2000 Å in a dried state in case of steer hide. These fibrils are assembled parallel to each other to form the so-called "fiber". The "fiber" has a diameter of 2—6 μ in a dried state in case of steer hide. Further, these fibers are assembled parallel to each other to form a bundle-like structure that is termed "fiber-bundle". The "fiber" dispersion is treated under a controlled condition of alkali treatment so that the telopeptide moieties at the end of the collagen molecules may be removed at the same level as the solubilization with an enzyme or alkali, while the rate of diffusion and dissolution of collagen within eyes may be optionally controlled by adjustment of the level of glutaraldehyde treatment. In the procedures according to this method, the natural molecular arrangement of collagen in the "fiber" retain as it stands which leads to an effective control of slow-release of a drug depending upon the sort of drugs to be used and then makes it feasible to utilize the drug as an ophthalmic pharmaceutical preparation having slow-releasing property.

The drug or medicament which may be employed in the present process may be any of water-soluble and insoluble ones. As ophthalmic medicaments are mydriatics such as atropine, homatropine, scopolamine, hydroxyamphetamine, phenylephrine, cyplegin, lachesine, cyclopentolate, and tropicamide and miotics such as pilocarpine, physostigmine, carbamylcholine, demecarium and phospholine iodide. Antibiotic substances may be used, for example, β-lactam group antibiotics such as caphalexin or cephalothin; tetracycline group antibiotics such as tetracycline; aminoglucoside group antibiotics such as streptomycin, kanamycin, ribostamycin, dibekacin, aminodeoxykanamycin and macrolide group antibiotics such as midecamycin other agents include anti-inflammatory corticosteroids such as cortisone, hydrocortisone, betamethasone, dexamethasone, predonisolone, fluorocortolone and triamcinolone, β-receptor blocking agents such as propranolol, pindolol, alprenolol, bufuetolol, bupranolol, bunitrolol, practolol and oxyprenolol, and idoxuridine, epinephrine, glycyrrhizic acid, vidarabine and other suitable medicaments as well as their derivatives such as salts, and covalent derivatives, e.g., esters or amides active medicaments may be employed.

With regard to a controlled pharmaceutical preparation using collagens, there has been disclosed in Japanese Patent laid open publication No. 42025/1975, the preparation of a gel- or film-like pilocarpine-containing pharmaceutical preparation, which is characterized in that solubilized telopeptide-free collagens are contacted and mixed with the intraocular pressure depressant pilocarpine. Collagen can be dissolved in water with a limited maximum concentration of about 20%, but the viscosity of the collagen solution is extremely high. Consequently, the practicable critical concentration is 5% even at the highest for the preparation of film- or gel-like compositions obtained according to the disclosure in the above Japanese Patent (laid open publication No. 42025/1975). In addition, when a gel form is to be prepared according to the above process, steps of cross linkage with γ rays or ultraviolet rays and of subsequent washing with water are further required, while, in case of a film form obtained by the so-called air drying method, there is an economically difficult problem in the mass production thereof, as a prolonged time is required for drying and the like.

In the present invention, there may be employed a precipitate having a collagen con-

centration of 30—70 percent by weight, which can be btained by concentrating by centrifugation or filtration under a pressure of the precipitate formed at the isoionic point of the collagen solubilized by use of the above-depicted enzyme or by alkali method. The isoionic point is pH 7—9 in case of the collagen solubilized by the enzyme method or pH 4.8 in case of the collagen solubilized by the alkali method. Moreover, a drug may be incorporated into the said precipitate at a concentration of 5—20% by weight, well kneaded, and then the resulting product as it is may be employed as an ointment. Moreover, to the mixture of collagen and drug may be admixed, for instance, 5—20% by weight of a lower polyhydric alcohol such as polyethylene glycol, propylene glycol or ethanol. This may be done both when the preparation is to be provided in the form of an ointment and in the form of a moulded preparation. In the latter instance the composition has improving moldability and the resulting mixture can be extruded to a rod shape or formed into tablets by a tabletting machine and, if necessary, dried for use. According to the present process, there may be such advantageous characteristics in that the steps subsequent to mixing of a drug are simplified, drying is easily effected owing to a lower water content and a higher collagen concentration and the amount of a drug may easily be held constant. In use, there may be employed an ointment form, but an aqueous collagen solution having an extremely high viscosity may also be employed and, when instilled in eyes, shows a higher retention of a medicament in comparison with the commonly applied eye drops.

This invention will be more fully illustrated by the following examples.

Example 1

Telopeptide-free collagen which was prepared and purified by a method for the solubilization of insoluble collagen such as the one disclosed in British Patent 1,119,432 was precipitated at pH 8 and concentrated under centrifugation.

To 100 g of the precipitate with a collagen content of 30% was added a solution of 4 g of pilocarpine hydrochloride in 25 ml of water and 3 g of polyethylene glycol 400 which reduces the viscosity of the collagen precipitate to effect an easy molding and flexibility of the final product.

The mixture was well kneaded and extruded through a nozzle with an inner diameter of 2 mm and dried. After drying, the rod had a diameter of 1 mm. The rod was cut into 6 mm lengths (each having a weight of 6 mg and a pilocarpine content of 0.7 mg). Five of the cut rods were placed in 200 ml of Ringer's solution and the amount of pilocarpine released into the solution was measured. The results are illustrated in Fig. 1, of the accompanying drawings.

As can be seen from Fig. 1, the curve formed by plotting pilocarpine release as percentage against time is an integral exponential curve and may be represented by the following equation (1) where the release percentage and time are defined as Y and t, respectively.

$$Y=100\ (1-\mathrm{Exp}(-kt)) \qquad (1)$$

wherein k is a release constant, a constant value is g—value in a particular preparation, and, when calculated from experimental values shown in Fig. 1, 0.035 min^{-1}. Alternatively, it may be calculated from the equation

$$T_{1/2}=0.693/k \qquad (2)$$

The $T_{1/2}$ means a time required for release of 1/2 of a medicament in a preparation and is referred to hereinafter as "half-life period of release". The $T_{1/2}$ is also a constant value for a particular preparation. The half-life period of release in this Example was 19.8 minutes.

As pilocarpine hydrochloride is very easily soluble in water, all 3.5 mg of the powdery hydrochloride can be instantly dissolved when it is added to water as a solid. Therefore, the results illustrated in Fig. 1, reveal that the collagen rod acts as a good control on the release of pilocarpine.

One rod of 6 mm length was inserted into the inside of lower eyelids of rabbit. After 7 minutes, contraction of pupils initiated and continued over 6 hours as shown in Figure 2 of the accompanying drawings. Also, pupillary contraction caused when a 0.6% pilocarpine conventional eye drop was instilled in eyes of rabbit is shown in Fig. 2. In case where the conventional eye drops were instilled in eyes, maximum pupillary contraction occurred in 20 minutes and then eyes were restored to normal as before in 80 minutes, whereas, in case of insertion of collagen rod, maximum contraction occurred in 2 hours and contraction continued up to 6 hours. In case where the pilocarpine-containing collagen rod with 6 mm in length was applied to a patient suffering from glaucoma, one insertion caused the lowered intraocular pressure continuous even more than 1 day. Considering the results of the above test of pilocarpine release in Ringer's solution as shown in Fig. 1, together with the results of the above sustained release test in animals and human patients, one can understand that the preparation may have a satisfactory prolonged effectiveness in a living body and sufficiently achieve the purpose of this invention, if only the half-life period of release, $T_{1/2}$, is set to around 20 minutes. The collagen rod was dissolved away in 1 day.

Example 2

Telopeptide-free soluble collagen prepared by the so-called alkali method disclosed in British Patent 1,192,314 was precipitated at pH 4.8 and centrifuged at 20,000—30,000 r.p.m. 100 g of the precipitate having a collagen concentration of 50% was well kneaded with a solution of 7 g of pilocarpine hydrochloride in 10 ml of water and further 5 g of ethanol for better molding. The kneaded stock was compression-molded by a die which was 8 mm long, 2 mm wide and 1 mm thick, and then dried. The dried molding had a length of 7 mm, a width of 1.2 mm, a thickness of 0.5 mm and a weight of 6 mg. This molding had a pilocarpine release constant k of 0.028 min^{-1} in 200 ml of Ringer's solution and a half-life period of release of 24.75 minutes and showed nearly the same level of release time as that obtained in Example 1. This molding when applied to a patient suffering from glaucoma exerted a sustained lowering of intraocular pressure for more than 1 day. The collagen rod was dissolved away in 6 hours.

Example 3

The butt region cut from a steer hide was dehaired and washed with water. The grain and flesh layers were further removed and the corium layer thus obtained was cut into 10 cm squares. The pieces were rinsed with a 10% aqueous sodium chloride solution and washed well with water. They contained insoluble collagen at 25% by weight. 400 g of the material was soaked in 600 ml of an aqueous solution containing 20 g of caustic soda, 160 g of sodium sulfate and 20.7 g of a 30% monomethylamine aqueous solution for 7 days at 20°C., desalted by washing with water and the pH was adjusted to 6.0 with hydrochloric acid.

The so treated material were finely divided through a mincing machine with the holes of a diameter of 7 mm and then completely ground through a disperse mill. The resulting dispersion was suspended in 10 l of a 15% w/v aqueous solution of sodium sulfate and the pH was adjusted to 8.5 by the addition of 2N sodium carbonate. Tanning was conducted with 200 ml of a 25% glutaraldehyde aqueous solution at 25°C for 5 hours. After washing with water, the whole dispersion was made up to 5 l, adjusted to pH 3.0 with 2N hydrochloric acid and again ground through a disperse mill. The "fiber" dispersion thus obtained was adjusted to pH 5.0 and concentrated with centrifugation to give a dispersion having a collagen content of 50%. 100 g of the resulting dispersion and a solution of 5 g of ribostamycin sulfate in 25 ml of water were thoroughly mixed and kneaded. The resulting mixture of collagen dispersion and ribostamycin sulfate was compression-molded in the same manner as in Example 2 and dried. The dried weight was 6 mg, containing 0.6 mg of ribostamycin sulfate. 10 pieces of the molded product were placed in 200 ml of Ringer's solution and an amount of ribostamycin sulfate released into the solution was measured at 37°C. The results are shown in Fig. 3 of the accompanying drawings, in which the curve is similar to that in Fig. 1 and may be represented by the equation (1) as shown for Fig. 1. Release constant k of this molded product was 0.0166 min^{-1}, while a half-life period of release was 41.7 minutes and rather longer as compared with that of pilocarpine in Example 1. This molded product must have a far more prolonged duration in a living body than the rod of Example 1 has. Since ribostamycin sulfate is very easily soluble in water, all 6 mg of the powdery sulfate can be instantly dissolved in water. Therefore, the molded product from the collagen fiber dispersion, as can be seen from the results shown in Fig. 3, controls the elution of ribostamycin sulfate so sufficiently as to sustain its action. This molded product when inserted in the lower eyelids of rabbit was dissolved away in 1 day.

Example 4

Solubilized collagen was prepared according to the so-called alkali method disclosed in British Patent 1,192,314. A precipitate having a collagen content of 30% was prepared at pH 4.8. 100 g of the precipitate and a suspension of 0.6 g of powdery dexamethazone sulfate sodium salt in 20 ml of water were well mixed and kneaded. The kneaded mixture was then extruded through a nozzle with an inner diameter of 2 mm and dried in the same manner as in Example 1. The dried rod had a diameter of 1 mm. The rod with 6 mm in length weighed 5.5 mg per one, containing 0.1 mg of dexamethazone sulfate sodium salt. Release of dexamethazone sulfate sodium salt from the rod was measured by the use of 200 ml of Ringer's solution as done in the foregoing Examples. As a result, a release constant k was 0.021 min^{-1} and a half-life period of release was 33 minutes, which proved to be so satisfactory as to exert a prolonged action in a living body.

Example 5

Solubilized collagen was prepared according to the so-called alkali method disclosed in British Patent No. 1,192,314. A precipitate having a collagen content of 30% was prepared at pH 4.8. 100 g of the precipitate and a suspension of 1 g of idoxuridine in 20 ml of water were well mixed and kneaded. The kneaded mixture was then extruded through a nozzle with an inner diameter of 2 mm and dried in the same manner as in Example 1. The dried rod had a diameter of 1 mm. The rod of 6 mm length weighed 5.5 mg and contained 0.17 mg of idoxuridine. Release of idoxuridine from the rod was measured by the use of 200 ml of Ringer's solution. As a re-

sult, a release constant k was 0.008 min^{-1} and a half-life period of release was 86.6 minutes, which corresponded to satisfactory results to sustain the duration of action in a living body.

Reference Example

Into cul-de-sac in one eye each selected at random from 7 patients suffering from primary open angle glaucoma was inserted the pilocarpin-containing collagen rod of 10 mm length prepared in Example 1 and then changes in intraocular pressure and pupillary size were observed as the time lapsed. After two weeks, 100 μl of 4% pilocarpine was instilled in eye and changes in intraocular pressure and pupillary size were observed.

Changes in intraocular pressure and pupillary size of treated eyes with time are as shown in Figs. 4 and 5 of the accompanying drawings, together with those of non-treated eyes as a control.

When applied in the molded form of collagen, a markedly sustained effect was observed as compared with case of instilling conventional pilocarpine drops in eyes, and a statistical significance (P) was found to be 0.05 between two cases at both 8 hours and 24 hours after application. In particular, the effect of pilocarpine on pupil and intraocular pressure wore off after 24 hours when the drops were installed to eyes, whereas significant contracted pupil and lowered intraocular pressure were observed even after 24 hours when applied in the molded form of collagen.

WHAT WE CLAIM IS:—

1. An ophthalmic pharmaceutical preparation having a slow-releasing property which comprises a telopeptide-free collagen precipitate obtained by precipitation of a solubilized collagen solution at isoionic point or a telopeptide-free collagen "fiber" dispersion obtained by treatment of a collagen "fiber-bundle" dispersion with an aldehyde, and one or more ophthalmic drugs.

2. A pharmaceutical preparation according to claim 1 wherein said collagen precipitate has a collagen content of 30—70% by weight.

3. A pharmaceutical preparation as claimed in claim 1 or 2, wherein a lower polyhydric alcohol is incorporated in an amount of 5—20% by weight with respect to collagen.

4. A pharmaceutical preparation according to claim 3, wherein the alcohol is selected from polyethylene glycol, propylene glycol and ethanol.

5. A pharmaceutical preparation according to any preceding claim, wherein said ophthalmic drug or drugs is selected from mydriatics, miotics, antibiotics, anti-inflammatory cortico steroids, β-receptor blocking agents, idoxuridine, epinephrine, glycyrrhizic acid and vidarabine.

6. A pharmaceutical preparation according to any preceding claim, wherein said preparation is in molded form.

7. A pharmaceutical preparation according to claim 6, wherein said preparation is a molded rod.

8. A pharmaceutical preparation according to any one of claims 1, 2, 3, 4 or 5, wherein said preparation is an ointment.

9. A pharmaceutical preparation according to claim 1, 2 or 5, wherein said preparation is a solution.

10. A pharmaceutical preparation according to claim 1, substantially as hereinbefore described with reference to any of Examples 1 to 5.

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Fig.1

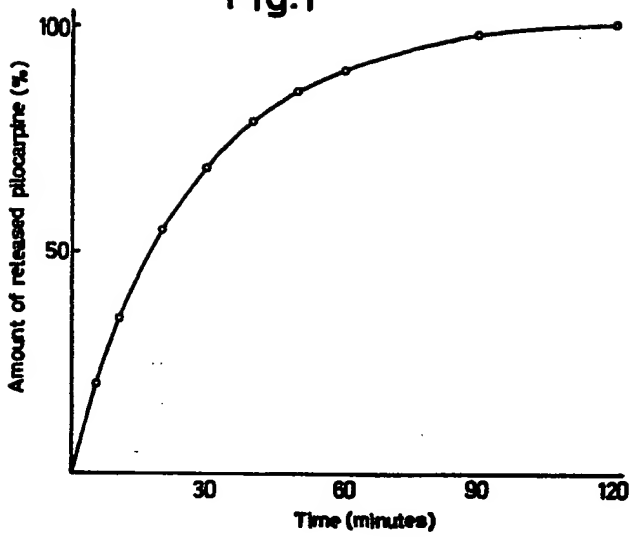

100
50
Amount of released pilocarpine (%)
30
60
90
120
Time (minutes)

Fig.2

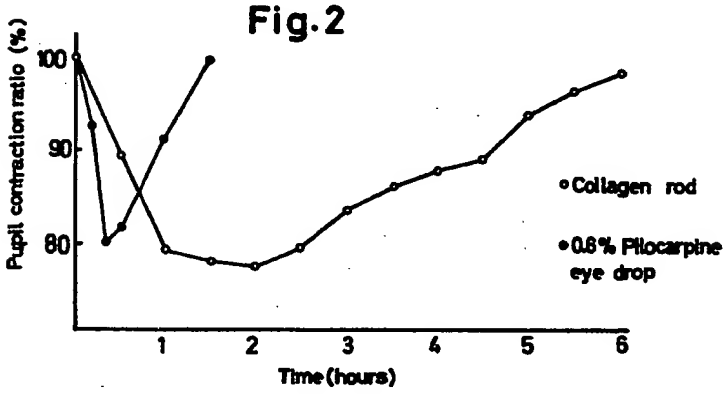

100
90
80
Pupil contraction ratio (%)
1
2
3
4
5
6
Time (hours)
Collagen rod
0.8% Pilocarpine eye drop

Fig.3

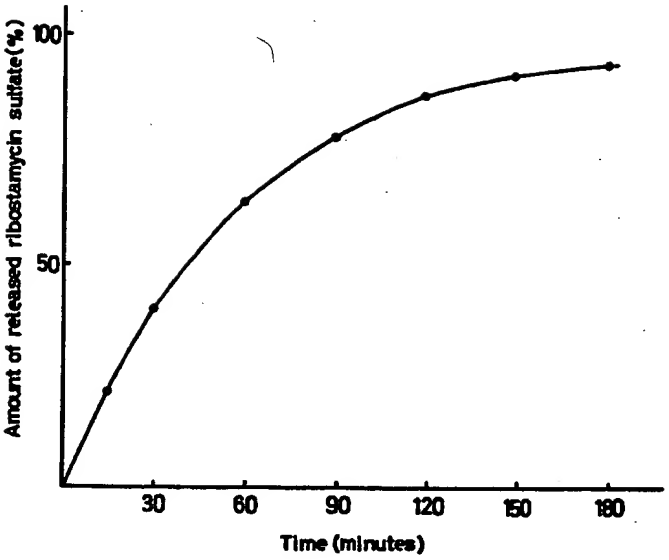
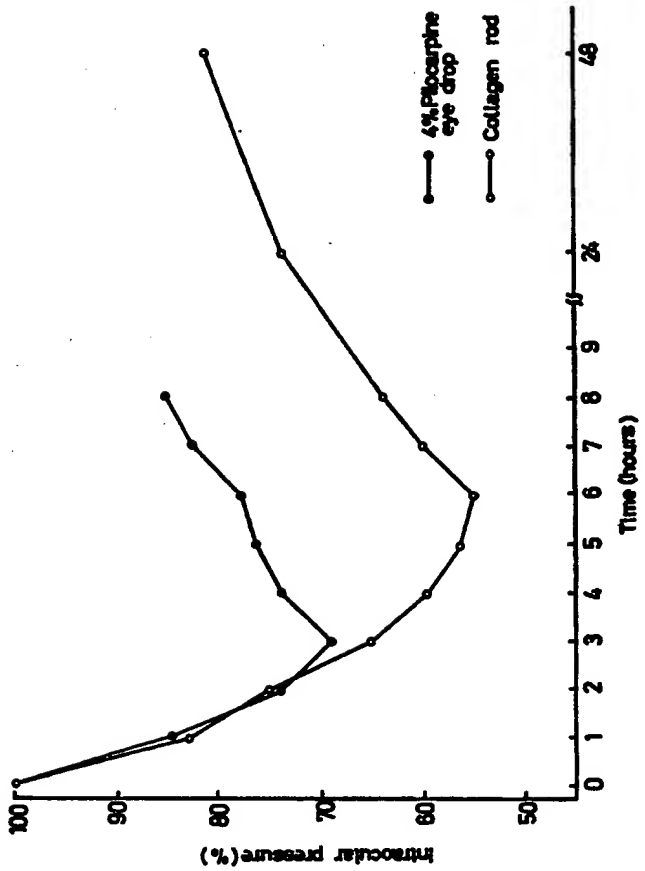

Amount of released ribostamycin sulfate(%)
100
50
30
60
90
120
150
180
Time (minutes)

Fig.4


4% Pilocarpine eye drop
Collagen rod
Time (hours)
0
1
2
3
4
5
6
7
8
9
24
48
intraocular pressure (%)
100
90
80
70
60
50

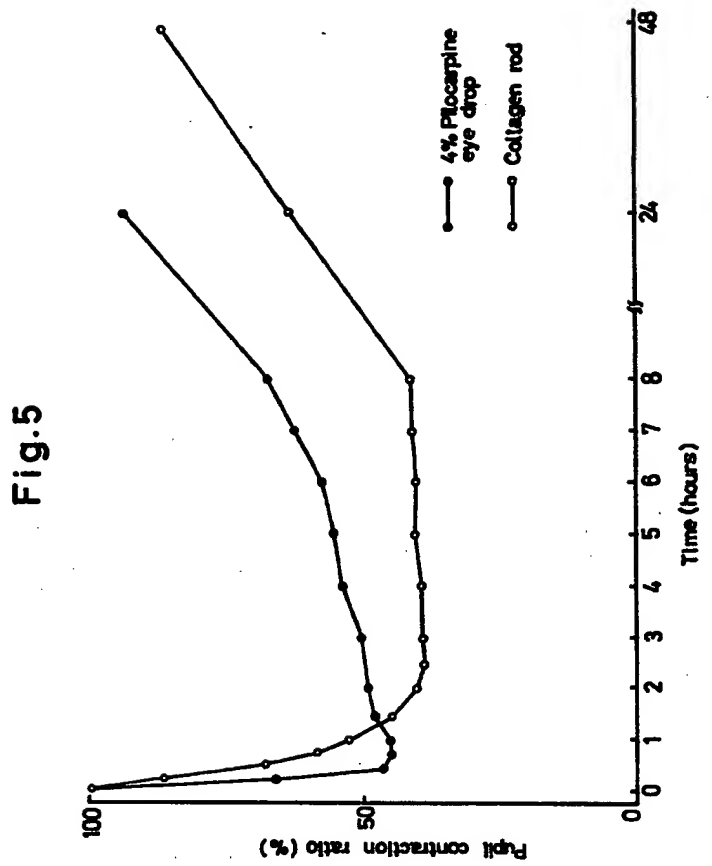

4% Pilocarpine eye drop
Collagen rod
Pupil contraction ratio (%)
100
50
0
0 1 2 3 4 5 6 7 8
24
48
Time (hours)

Fig.5